Fipronil: mechanisms of action on various organisms and future relevance for animal models studies

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Abstract. Because insects had developed resistance to several insecticides, today, neonicotinoids and fiproles are used to combat pests. The difference between this two classes of insecticides is that fipronil acts by inhibiting the receptors of nervous cells, while neonicotinoids perturbs the neuronal transmission. The suitable properties of fipronil make that its use to be more obvious on the pesticide market. Even if it is known that this insecticide has a direct target, there are cases when its effects are negative on other organisms. In the last period, it appeared a lot of reports describing the consequences of his use not only regarding insects. Vertebrates and even humans are exposed to its bad influence. The present paper contain a description of fipronil discovery, mechanism of action and some of the main effects which occur after administrating it, as well as, relevance for animal models studies.

Keywords. Insecticide, fipronil, GABA, ecological risk, neurotoxicity, vertebrates, humans, autism spectrum disorder

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Introduction

Nowadays the use of insecticides by humans is more and more pronounced worldwide. These chemicals which were developed for many years had a great influence on people's lifestyle. When a farmer observes that his crop is going down because the negative effect of insects, he tries to fight with them using various methods starting from removing the affected or infested leaves to apply on plants an adequate substance for certain pests. Usually, people do not think about the indirect effect of that substance and, many times, the structure of soil, the composition of the water or the organisms which are living in these habitats are affected. For example, macroinvertebrates as mosquitoes are affected due to the fact that their diet with aquatic organisms is perturbed. Aquatic organisms from microbial communities are influenced indirectly by the presence of the insecticide in the water, insecticide who arrived there through the direct use of it by humans (Muturi *et al*., 2017). Because of their long resistance in environment, doses of insecticides can be taken or ingerated by insects or even humans like eating fruits which contains remains of the substances spreaded against pests or contamined pollen used by bees to feed the larvae (Sánchez-Bayo *et al*., 2013). These are only two examples about what is happening when those substances are used and how the balance between biotope and biocenosis is disrupted.

Due to the large number of chemicals which are the active substance in various pests products, insecticides have, in general, specific targets. However, their differents between provenance, chemical structures and mechanism of action leads to a certain organization of them (Gupta and Milatovic, 2014). Evidence suggests that some insecticides are inductors of neuropsychiatric features of some nervous system disease. Natural or synthetic, insecticides are widespread in different forms, extracted from plants like rotenone, an alkaloid from Derris, Lonchocarpus, Tephrosia, and Mundulea genus, known for determining Parkinson's disease symptoms and used for fish extermination, or produced by humans through various combination of raw materials, the last are dominating the market (Gupta and Milatovic, 2014; Robea *et al*., 2018).

The most known synthetic insecticides are: organochlorides, organophosphates, carbamates, pyrethroids, neonicotinoids and fiproles (Ware and Whitacre, 2004; Gupta and Milatovic, 2014). In general, an insecticide acts on insect nervous system which leads to an anormal function of neurotransmitters, moment when the insecticide molecules binds in the places of a neurotrasmitter sites, more exactly dereglates the function of specific cellular channels (Bloomquist, 1999).

Fipronil (5-amino-1-[2,6-dichloro-4(trifluoromethyl)phenyl]-4-[(1R,S)-

(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile) is a member of fiproles who acts on chloride channels blocking the activation of GABA (Bobé *et al*., 1998; Bloomquist, 1999; Mohamed *et al*., 2004; Ware and Whitacre, 2004; Gwaltney-Brant, 2013; Gupta and Milatovic, 2014; Oberemok *et al*., 2015).

At its beginnings, fipronil was discovered and promoted by a chemical and pharmaceutical company from France named: Rhône-Poulenc (EB, 2003; Gunasekara *et al.*, 2007; Narahashi, 2010; Hodgson, 2012). After its dicovery, fipronil started to be utilised as a tool against a several classes of insects (Page, 2008). Today it is produced by a german company, Badische Anilin und Soda Fabrik (BASF) as a chemical suspension packed in products with different names for plant protection, urban pest and fish control or as a veterinary instrument (Simon-Delso *et al*., 2014; BASF, 2018). More often, utility of fipronil is observed in dogs and cats treatments when it is administrated locally, on skin, to fight against ticks, ear mites or fleas (Sternesen, 2004; Page, 2008; NPIC, 2009; Gwaltney-Brant, 2013; Gupta and Milatovic, 2014). It was used on rabbits but the vets do not recommend its usage because of the adverse effects like: depression or a reduced appetite (Gupta and Milatovic, 2014; Varga, 2014).

Fipronil – Mechanisms of action

Fipronil is a phenylpyrazole compound which is well-known for disturbing the ligand-gated chloride channels from the cell membranes of insects (Bloomquist, 2003; Narahashi, 2010). The chloride channels are responsible with the hyperpolarization of the potential membrane, with other words, it favors the entrace in the cell of chloride ions (Bloomquist, 1999; Bloomquist, 2003; Mohamed *et al*., 2004). Even if the principal target is the nervous system, these channels can be affected in muscles or kidney too (Bloomquist, 2003). Binding the molecules of insecticides to the specific sites of chloride channels leads to the inhibition of GABA and glutamate neurotransmitters and, as a consequence, it determines the hyperexcitation of central nervous system (Bloomquist, 1999; Bloomquist, 2003). The entire mechanism is represented in Fig. 1.

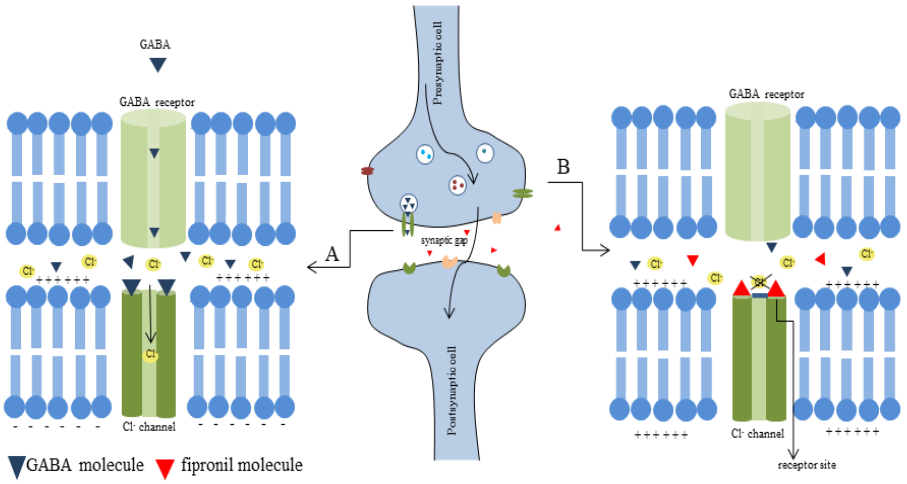

Figure 1: The mechanism of fipronil, A: normal state, B: the activity of fipronil on GABA chloride channels.

Ecological risk

Currently, the negative effect of insecticides on indirect organisms represent a major problem for researchers considering the imposibility to manage the unpleasant issues after exposure to them. According to several reports made by the Environmental Protection Agency (2008) from United States, it were identified a series of ecological risk reflected on freshwater, estuarine, marine invertebrates and vertebrates, whose consequences depends if it is an acute or an chronic poisoning. In the Netherlands, fipronil was used illegally to eradicate the poultry red mite from the cabbage, leek and onions crops. This fact conducted to a damaging process because the aquatic crustaceans and insects populations present in the surface water became more sensible to this compound (Gunasekara *et al*., 2007; Tennekes, 2018). The potential role of fipronil depends also by its metabolites who are a result of various processes like: hydrolysis (fipronil-amide), reduction (fipronil-sulfide), oxidation (fipronil-sulfone) and fipronil-desulfinyl, a product of photolysis (Zhao *et al*., 2005; Gunasekara *et al*., 2007; Lin *et al*., 2009; Simon-Delso *et al*., 2014; Teerlink, 2017). The chemical formulas of fipronil and its metabolites are represented in Fig. 2.

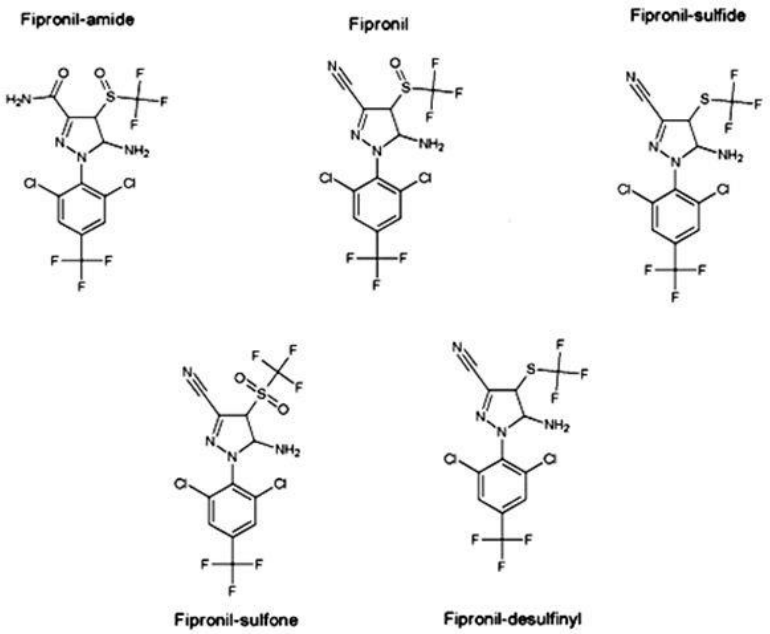

Figure 2: Structure of fipronil and its metabolites (after Tennekes, 2018).

Fipronil can be degraded by light, water or by microorganisms from soil biota (Bobé *et al*., 1998; Lin *et al*., 2009; NPIC, 2009; Simon-Delso *et al*., 2014). In soil it is descomposed in small molecules by the microorganisms which live there (NPIC, 2009). The principal degraded product is fipronil-sulfide, a result of reduction process (Gunasekara *et al*., 2007). In one report made by Lin *et al.* (2009), it was demonstrated that the degraded products of fipronil have a higher persistence under aerobic or anaerobic conditions than fipronil. Aproximatelly 125 days are necessary to be removed from the soil and that because its metabolites are adherent to the soil particles (Lin *et al*., 2009; NPIC, 2009). Also, physiochemical properties of these compounds and the organic carbon content from soil influence the sorption of them (Ying and Kookana, 2001; Singh *et al*., 2014).

Due to its low solubility in the water, fipronil persists a long period in the aquatic habitats and that's why it is considered more lethal on aquatic organisms (Ying and Kookana, 2001; Gunasekara *et al*., 2007; Tennekes, 2018). In water, fipronil is converted in fipronil-desulfinyl through photolysis (Gunasekara *et al*., 2007). There are a lot of studies all over the world which triggers an alarm semnal to take care of fipronil doses released during plant treatments (Tennekes, 2018).

Exposure of organisms to fipronil: possible neuro-relevance

Despite the fact that fipronil is used against certain pests, sometimes it affects non-target organism. Fipronil, fipronil sulfone and fipronil sulfide are

known as a toxic enhacer to aquatic organisms which are not targeted to be removed from that area and have aproximately the same actions (Wu *et al*., 2014). Fipronil sulfone is more powerful to block the $GABA_A$ receptors from mammals than fipronil (Zhao *et al*., 2005).

Being an insecticide is evident that the principal target of it are insects. According to Gunasekara *et al*. (2007) some of the fipronil lethal doses for insects are: 1.54, 0.43 and 23.0 µg/L for several species of mosquitoes, 0.42 µg/L for midges and for one bee is 4-6.2 ng. After insects, aquatic organisms are the second target of fipronil. 16 µg/L of fipronil is the lethal dose for Daphnia pulex, 0.14 µg/L for mysid shrimp and 130 µg/L for sheephead minnow (Gunasekara *et al*., 2007). In one report, it was evaluate the lethal dose of fipronil on red swamp (Procambarus clarkii) and white river crayfish (Procambarus zonangulus) taking in attention the fact that fipronil can be consumed simultaneously with the organic sediment. 14.3 and 19.5 µg/L were the lethal doses of fipronil for red swamp and white river crayfish (Schlenk *et al*., 2001).

The great majority of the studies which were conducted on laboratory animals concluded that fipronil action is, mainly, on growth, development and reproduction of the organisms (Gibbons *et al*., 2014). Also, the unfavorable effect of fipronil is observed on nervous system (Hainzl and Casida, 1996; Gibbons *et al*., 2014; Gupta and Milatovic, 2014). For instance, in one report made by some researchers, it was found that a certain dose of this insecticide can dereglated the thyroid hormone, induce seizures or even death (NPIC, 2009; Gupta and Milatovic, 2014). Depending the mode of administration and the dose, its effects are variable (Gunasekara *et al*., 2007). Tremors, convulsions and hunched posture have been observed on rats when fipronil was administrated orally (Mohamed *et al*., 2004). The same symptoms were observed after inhalating this substance (Gupta and Milatovic, 2014). If it is a dermal exposure, for example, rabbits are more affected than rats (Gupta and Milatovic, 2014, Varga, 2014). In Table 1 are

presented some reports with species of vertebrates, doses of fipronil and the main effects which were observed by researchers.

Table 1: The effects of fipronil on vertebrates.

Taxon and species	Concentration	Mode of administration	Effects	References
		Fish		
Zebrafish, *Danio rerio*	0.33 mg/L	Dissolved in water	Notochord degeneration, locomotor defects	Stehr *et al.*, 2006
	0.4 mg/L	Dissolved in water	Body length reduction, spine bending	Yan *et al*., 2016
	0.4 and 0.8 mg/L	Dissolved in water	Anxiety, swimming performance perturbed, lipid peroxidation increased	Wang *et al*., 2016
Fathead minnow, *Pimephales promelas*	0.14 mg/L	Dissolved in water	Impaired swimming	Beggel *et al*., 2010
	0.03 mg/L	Dissolved in water	Changes in gene transcription	Beggel *et al*., 2012
Silver catfish, *Rhamdia quelen*	0.0002 mg/L	Dissolved in water	Erythrocyte damage	Ghisi *et al*., 2011
		Birds		
Bobwhite quail, *Colinus virginianus*	0.11mg/Kg	Injected	Body mass reduction, absence of apetite, developmental abnormalities	Kitulagodage *et al*., 2011b
Chicken, *Gallus gallus domestica*	37.5 mg/Kg	Injected	Developmental abnormalities	Kitulagodage *et al*., 2011a
		Mammal		
Rat, *Rattus norvegicus*	280mg/Kg	Injected	Low changes of pregnancy	Ohi *et al*., 2004
	20mg/Kg/day	Injected	Weight gain reduction	Tingle *et al*., 2003

People are exposed to fipronil through accidental contact, acute and chronic events or when it is about a suicide tentative (Jeyaratnam, 1990; Chodorowski and Anand, 2004). In all these cases, fipronil acts on $GABA_A$-gated chloride channel determining hyperexcitability (Mohamed *et al*., 2004; Narahashi, 2010). Headache, seizures, convulsions, pneumonia or

death were some of the symptoms of fipronil ingestion (Mohamed *et al.*, 2004; Gupta and Milatovic, 2014). Because fipronil is known to dereglate the function of GABA neurotransmitter and an abnormal function of it was found as a symptom of autism spectrum disease, this fact leads to conclude that fipronil can be used to modelate an animal model for autism (Pessah, 2008).

In some reports, it was highlighted the neurotoxic effect of fipronil. For the first time, the blocking activity of fipronil was tested on a cellular line of Drosophila (Ikeda *et al*., 2004). The main effect consisted in appearance of some changes in the activity of ion channels, more exactly, fipronil was responsable with shortening the period of channel opening (Grolleau and Sattelle, 2000; Ikeda *et al*., 2004). Furthermore, Ikeda *et al.* (2001) studied the activity of ion channels from dorsal root ganglion neurons of rats. 1.66 +/- 0.18 μM of fipronil was the estimated dose needed to block the $GABA_A$ receptors from dorsal root ganglion neurons of rats (Narahashi, 2010).

Conclusions

Fipronil is an insecticide which exerts an neurotoxic effect especially on insects by blocking the GABA-chloride channels. It and his metabolites are highly toxic to aquatic organisms according to several reports. All the information presented in this report sustains that fipronil is capable to exert direct and indirect influences on insects, aquatic and terrestrial organisms. Further studies are needed to reduce the risk of accidental exposure and to make a corelation between fipronil and autism and to validate if fipronil can be a succesfully tool for studying autism spectrum disorder in an animal model.

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